The Karyotypes of Megophryinae (Pelobatidae) with a Discussion on their Classification and Phylogenetic Relationships

DING-QI RAO AND DA-TONG YANG

Kunming Institute of Zoology, Academia Sinica, Kunming 650223,China

Abstract. -The karyotypes of nine Megophryinae species from China were studied. *Megophrys omeimontis* Liu has 2n=26 with 5 large, 1 moderate and 7 small pairs, 8M+3SM+2ST, NF=52, 1 secondary constriction in 6p. *Megophrys giganticus* Liu has 2n=26 with 5 large and 8 small pairs, 5M+5SM+2ST+1T, NF=48. *Megophrys minor* Stejneger has two different forms. One is 2n=26 with 6 large and 7 small pairs, 8M+2SM+2ST, NF=52; the other is 2n=30 with 6 large and 9 small pairs. The large group is identical to that of the 2n=26 form, 8M+2SM+3ST+2t, NF=56. *Megophrys kuatunensis* Pope has 2n=24 with 6 large and 6 small pairs, 5M+2M(SM)+2SM+3ST. *Megophrys lateralis* (Anderson) from Tengchong, Yunnan has 2n=26 with 6 large and 7 small pairs, 8M+3SM+2ST, NF=52. *Megophrys lateralis* (Anderson) from Hekou, Yunnan has 2n=26 with 6 large and 7 small pairs, 5M+1M(SM)+4SM+2ST+1T. *Megophrys parva* Boulenger has 2n=26 with 6 large and 7 small pairs, 4M+5SM+1SM(ST)+3T, one secondary constriction 1Q; few metaphases with 2n=26+1 and 4 pair of T chromosomes. *Megophrys palpebralespinosa* Bourret has 2n+26 with 5 large and 8 small pairs, 5M+4SM+1SM(ST)+2ST+1ST(T). *Megophrys daweimontis* sp. nov. Has 2n=26, 5 large and 8 small pairs, 6M+3SM+1SM(ST)+1ST+1ST(T), one secondary constriction in 6Q. *Ophryophryne microstoma* Boulenger has 2n=26 with 6 large and 7 small pairs, 7M+1M(SM)+2SM+1ST+T. The karyotype of *O. pachyprocta* Kou resembles that of *O. microstoma*.

Karyotypes of the Megophryinae can be divided into 5+8 and 6+7 types. The karyotype of *M. omeimontis* appears to be an intermediate between 5+8 and 6+7. Combining the cytological, morphological and ecological characters, the classification of Megophryinae can be reviewed. The genus *Atympanophrys* is probable only needed to verify its main generic character. *Brachytarsophrys and Ophryophryne* are valid genera. *Megophrys* can be divided into four groups: I. *M. giganticus*, II. *M. montana* and *M. nasuta*, III. *M. palpebralespinosa* and *M. daweimontis* sp. nov., IV. Other species generally with small-sized bodies and 6+7 karyotypes. *M. giganticus* should probable belong to the genus *Atympanophrys;* a new genus should be erected for groups III and IV, whereas *Megophrys* should refer only to group II. A new species, *M. daweimontis*, is described below.

Key words: Megophryinae, *Brachytarsophrys*, *Megophrys*, *Ophryophryne*, karyotype, classification, phylogenetic relationships, *Megophrys daweimontis* sp. nov.

Materials and Methods

Megophrys lateralis were collected from Tengchong, Yunnan in 1991 and Hekou, Yunnan in 1993; *M. omeimontis* and *M. minor* from Jingdong, Yunnan in June, 1990 and May, 1991; *M. giganticus* from Jingdong in May, 1991; *M. palpebralespinosa* and *M. daweimontis* sp. nov. From Kekou, Yunnan in July 1993; *M. parva* and *Ophryophryne pachyprocta* from Mengla, Yunnan in May 1993; *M. kuatunensis* from Chongan and Dehua, Fujian; : *O. microstoma* from Hekou, Yunnan in June 1992 and July, 1993. All the specimens were brought alive to the laboratory for karyotyping.

Specimens were injected intraperitoneally with colchicine at a dosage of 20 ug/g body-weight. After 24 to 36 hours the femur and tibia bones were removed and the ends cut off. The marrow cells were then washed out with 1% tri-sodium citrate solution, ground in hypotonic 0.64% KCl solution and fixed in 3:1 solution of methanol;acetic acid for two periods of 20 minutes each. Slides were prepared by the air-drying method and stained with 10% Giemsa PBS for about 20 minutes.

Chromosomes were divided into 4 groups according to the ratio of long arm/short arm in length: M= metacentric, the ratio is 1-1.7; SM= submetacentric, 1.7-3.0; ST= subtelocentric, 3.0-7.0; T= telocentric, more than 7.0. Chromosomes designated M, SM and ST possess 2 arms and those designated T possess only 1 arm. The fundamental number (NF) represents the total arm numbers for all chromosomes in one karyotype.

Results

The karyotype of *M. lateralis* from Tengchong, northwest Yunnan has 2n=26; 6 large and 7 small pairs; Nos. 3, 11 and 12 are SM, Nos. 8 and 13 are ST, the rest are M and NF=52. There are no satellite or secondary constrictions (Fig. 3c). This karyotype mostly agrees with that recorded by Wu (1987) for *M. lateralis*.

The karyotype of *M. lateralis* (Anderson) from Hekou, Yunnan is 2n=26; 6 large and 7 small pairs; Nos. 1, 5, 6, 10 and 11 are M, Nos. 2, 3, 7, and 12 are SM, Nos. 8 and 9 are ST, No. 13 is T, No. 4 can be M or SM and NF=52. Sometimes one secondary constriction is close to the centromere of the short arm of No. 2 (Fig. 3). This karyotype is different from that of specimens from Tengchong, Yunnan, especially in Nos. 2, 7, 9, and 13, and the location of the secondary constriction (it is in 5p in the results of Wu, 1987). We think that a morphological comparison should be made between the two populations as part of any evaluation of their specific status.

The karyotype of *M. omeimontis* from Jingdong, Yunnan has 2n=26; 5 large, 1 moderate, and 7 small pairs; Nos. 3, 7 and 12 are SM, Nos. 8 and 13 are ST, the rest are M and NF=52. One secondary constriction was near the base of the short arm of No. 6; there is no satellite (Fig. 3d). This karyotype agrees with that reported by Zheng and Wu (1989) for *M. omeimontis* based upon a population from Mt. Omei in Sichuan, but their results indicate that the secondary constriction was in the long arm of No. 6.

The karyotype of *M. giganticus* is 2n=26; 5 large and 8 small pairs are obvious; Nos. 2, 3, 4, 7 and 12 are SM, Nos. 9 and 13 are ST, No. 8 is T, the rest are M and NF=48. No satellite or secondary constrictions were found (Fig. 3e). This karyotype is not the same as that recorded by Li et al., (1993) for *M. giganticus*. Recognition of 5 large and 8 small pairs is the same as the karyotypes of *Brachytarsophrys*.

The karyotype of *M. minor* is variable in specimens from the same locality in Jingdong. One karyotype is 2n=26; 6 large and 7 small pairs; Nos. 7 and 13 are SM, Nos 8 and 12 are ST, the rest are M and NF=52. No satellite or secondary constrictions were found (Fig. 3a). The second karyotype is 2n=30; 6 large and 9 small pairs. The large chromosomes are identical to those of the 2n=26 form, but the small ones are different. Among them Nos. 7 and 10 are SM, Nos. 8, 12 and 13 are ST, Nos. 14 and 15 are T, the rest are M and NF=56 (Fig. 3b).

The karyotype of *M. kuatunensis* Pope is 2n=24; 6 large and 6 small pairs; Nos. 1, 2, 6, 10 and 11 are M, Nos. 5 and 8 are SM, Nos. 7, 9 and 12 are ST, Nos. 3 and 4 can be M or SM and NF=48. No satellites or secondary constrictions were found. It is the only karyotype where 2n=24 in the Megophryinae. Further study is needed to evaluate the taxonomy of this species (Fig. 3j).

We found the karyotype of *M. parva* Boulenger to be 2n=26; 6 large and 7 small pairs; Nos. 1, 2, 3, 4 and 5 are SM, Nos. 6, 7, 8 and 10 are M, Nos. 11, 12 and 13 are T, No. 9 varies between SM and ST and NF=46. One secondary constriction is near the centromere of the long arm of No. 1 (Fig. 3h). A few metaphases with 2n=26+1 and 4 pair of T chromosomes were found. This karyotype is different than that recorded by Li et al. (1993) from the same localities, especially in the arm length for large chromosomes, and the location of the secondary constriction (5q in the Li et al. result).

The karyotype of *M. palpebralespinosa* Bourret is 2n=26; 5 large and 8 small pairs; Nos. 1, 2, 4, 5 and 12 are M, Nos. 3, 6, 8 and 10 are SM, Nos. 7 and 13 are ST, No. 9 varies between ST and T, No. 11 can be SM or ST and NF=52 or 50. No satellites and secondary constrictions were found (Fig. 3g).

The karyotype of *M. daweimontis* sp. Nov. Is 2n=26; 5 large and 8 small pairs; Nos. 1, 2, 4, 5, 9 and 13 are M, Nos. 3, 6 and 8 are SM, No. 12 is ST, No. 7 varies between ST and T, and No. 10 can be SM or ST and NF -52 or 50. One secondary constriction is close to the centromere of the long arm of No. 6.; This karyotype is similar to that of *M. palpebralespinosa* (Fig. 3f).

The Karyotype of *Ophryophryne microstoma* Boulenger is 2n=26; 6 large and 7 small pairs; Nos. 1, 2, 4, 5, 6, 8 and 10 are M, Nos. 3 and 7 are SM, No. 9 is ST, Nos. 12 and 13 are T, No. 11 can be M or SM and NF=48. No satellites and secondary constrictions were found (Fig. 3k). This karyotype is similar to the 6+7 type of some *Megophrys* species.

The karyotype of *Ophryophryne pachyprocta* Kou resembles most closely that of *O. microstoma;* 2n=26; 6 large and 7 small pairs. The metaphase preparation for *O. pachyprocta* is not good enough to provide a plate here.

Discussion

Although *Megophrys omeimontis* is widely distributed in southwestern China, its type locality is Mt. Omei, Sichuan. In Table 1, its karyotypes come from two populations (Mt. Omei and Mt. Wuliang) and they are very similar: all are with 5 large, one moderate and 7 small pairs. One secondary constriction

occurs in the moderate sized No. 6. Two differences exist. Zheng and Wu (1989) considered No. 6 large, but it should be called moderate according to the relative length which they measured. The secondary constriction is in a short arm. Probably the two populations from Mt. Omei and Mt. Wuliang belong to one species. Fei et al. (1990) recognized the population from Mt. Wuliang as a separate species, *M. jingdongensis*.

The karyotype of *Megophrys giganticus* is of the 5+8 type, the same as those of the *Brachytarsophrys* species and *Atympanophrys shapingensis*. Further research is needed on the systematic positions of *M. giganticus* and *A. shapingensis*.

Megophrys kuatunensis is the only Megophryinae species discovered which has 2n=24. This karyotype is the 6+7 type since it has 6 large pairs of chromosomes. This species only occurs on Mt. Chongan and Mt. Daiyun, Dehua, Fujian Province, China, at the eastern edge of the distribution of the Megophryinae.

Three karyotypes occur in *M. lateralis* (see Table 1). Form I and II, based on the specimens from Tengchong, northwest Yunnan, are generally identical, but both of them are different from Form III in chromosome No. 2 and chromosome No. 13, and the location of the secondary constriction which is in the terminal short arm of chromosome No. 5 in form I, but in the base of the short arm of No. 2 in form III. Form III is based on the specimens from Hekou, southeast Yunnan, far away from Tengchong. The taxonomic status of the Hekou population needs additional attention after comparing their morphology to other populations.

Table 1. Karyotypes of some Megophryinae species.

Species	2n	NF	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
O. microstoma	26	48	M	M	SM	M	M	M	SM	M	ST	M	M/SM	T	T		
O. pachyprocta	26	?	6 pairs									7 pairs					
M. parva (I)	26	46	SM	SM	SM	SM	SM	M	M	M	ST/SM	M	T	T	T		
M. parva (II)	26	46	M	M	SM	M	M	M	SM	M	M	M	T	T	T		
M. lateralis (I)	26	52	M	M	M	M	M	M	M	ST	M	M	SM	SM	ST		
M. lateralis (II)*	26	52	M	M	SM	M	M	M	M	ST	M	M	M	M	ST		
M. lateralis (III)	26	50	M	SM	SM	M	M	M	SM	ST	ST	M	M	SM	T		
M. minor (I)	26	52	M	M	M	M	M	M	SM	ST	M	M	M	ST	SM		
M. minor (II)	30	56	M	M	M	M	M	M	SM	ST	M	SM	M	ST	ST	T	T
*M. boettgeri**	26	50	M	M	SM	M	M	M	SM	M	SM	M	SM	M	T		
M. kuatunensis	24	48	M	M	M/SM	SM	M	M	ST	SM	ST	M	M	ST			
M. omeimontis (I)*	26	52	M	M	SM	M	M	M	SM	ST	M	M	M	SM	ST		
M. omeimontis (II)*	26	48	M	M	SM	M	M	M	SM	M	T	M	M	SM	T		
M. palpebralespinosa	26	52/50	M	M	SM	M	M	SM	ST	SM	ST/T	SM	SM/ST	M	ST		
M. daweimontis sp. nov.	26	52/50	M	M	SM	M	M	SM	ST/T	SM	M	SM/ST	M	ST	M		
*M. shapingensis**	26	52	M	M	SM	M	M	M	SM	SM	SM	M	M	SM	ST		
M. giganticus (I)*	26	48	M	SM	SM	SM	M	M	SM	T	ST	M	M	SM	ST		

(Continued)

Species	2n	NF	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
M. giganticus (II)*	26	50	M	SM	SM	SM	SM	ST	M	M	M	M	M	M	M		
M. nasuta (I)*	26	50	M	SM	M	SM	M	M	SM	ST	SM	M	M	SM	SM		
M. nasuta (II)*	26	50	M	SM	SM	M	M	SM	M	ST	M	M	SM	M	SM		
*M. monticola**	26	?	5 pairs								8 pairs						
B. sp. nov. (I)*	28	48	M	SM	M	M	M	M	M	SM	T	T	M	M	T	T	
B. sp nov. (II)*	26	44	M	SM	M	M	M	M	M	M	T	M	T	T	T		
*B. feae**	26	50	M	SM	M	M	M	SM	M	M	SM	T	M	M	SM		
B. carinensis	?	?	5 pairs (?)								(?)						
Groups			Large Group								Smalll Group						

* M. lateralis (II) and M. shapingensis are from Wu (1987); M. boettgeri from Gao et al. 1990; B. sp. nov. (II) from Tan et al. (1987, as B. carinensis); M. omeimontis (II) from Zheng and Wu (1989); M. giganticus (II) and M. parva (II) from Li et al. (1993); M. nasuta (I) and M. monticola (synonym of M. montana) from Morescalchi et al. (1977); M. nasuta (II) from Schmid et al. (1987). M. lateralis (II) based on specimens from Tengchong, Yunnan; M. lateralis (III) based on specimens from Hekou, Yunnan; M. giganticus (I), B. sp. nov (I) and B. feae are from Rao and Yang (in press).

Two different forms of karyotypes of *Megophrys parva* are also shown in Table I; forms I and II are based on the specimens from Mengla, Yunnan. This species is more primitive in that it has 3 pairs of T chromosomes in both forms. It may be primitive because it is widely distributed in upper Burma and southwest Yunnan.

Although *Megophrys palpebralespinosa* occurs in Hekou, Yunnan, it is very close to *M. boettgeri* of Fujian in skin and color characters. They are very different in karyotype: the former has a 5+8 type karyotype while the later has a 6+7 type. *M. palpebralespinosa* has tubercles on the outer edge of the upper eyelids, folds on the back and a reddish inner palm tubercles, while *M. boettgeri* does not. They are geographically widely separated. This suggests that they are not closely related.

Megophrys daweimontis sp. nov. is like *M. palpebralespinosa* in having a 5+8 karyotype, tubercled upper eyelids and reddish inner palm tubercles, and both occur on Mt. Dawei.

It is interesting that *Megophrys palpebralespinosa* and *M. daweimontis* sp. nov. Have 5+8 type karyotypes. Generally the 5+8 type karyotype occur in species which are large-sized and possess a hidden tympanum, such as *M. nasuta*, *M. monticola* (synonym of *M. montana*), *M. giganticus*, *M. shapingensis* and *Brachytarsophrys* species. Species with small-sized bodies and a distinct tympanum generally have 6+7 karyotypes common in the Megophryinae. *M. palpebralespinosa* and *M. daweimontis* sp. nov. are relative small; body length is about 35 mm. In males and 40 mm. In females. Additional study of their taxonomic position is needed.

Karyotypes of *Ophryophryne microstoma* and *O. pachyprocta* are of the 6+7 type, the same as those of *Megophrys* species which are generally small-sized. These observations agree with Dubois (1980) who considered *Ophryophryne* a subgenus of *Megophrys*. However, on the basis of other characters, habitats and habits, which are different from other Megophryinae species, we recognize this genus as valid and separate. According to Kou (1985), *O. pachyprocta* has a bump above the anus. We think this bump is probably the protruding coccyx and *O. microstoma* also has a protruding coccyx. On the basis of this character, we think that the two species should be recognized as a single species.

The main differences among the species are shown in Table I. But another should be noted: *Megophrys boettgeri* has a secondary constriction in 6q and *M. shapingensis* also has one near the centromere of 1q.

Megophrys minor from Jingdong, Yunnan has various karyotypes, but we can't find anatomical differences between the different specimens with different karyotypes. Karyotype variation is common in Pelobatidae, such as *Leptolaylax pelodytoides* (Li et al., 1991). Because there are various karyotypes in the same species, it is difficult to identify a species according to its karyotype, especially for some widely distributed species, such as *M. lateralis*, *M. omeimontis* and *M. minor*. In contrast, some Pelobatidae have very stable karyotypes with simple chromosome arrangements and secondary constrictions, such as *Oreolaxax*, *Scutiger* and *Vibrissaphora*. The karyotypes of some genera are variable, such as *Leptolaylax* and some *Megophrys* species. Thus, karyotypes can be used to resolve some taxonomic problems in some genera, but not in others. In general, karyotypic variation in Megophryinae occur among small chromosomes, while the large chromosomes are relatively invariable. The karyotypes of Megophryinae species, which are similar in morphology and habitats, are relatively similar. So karyotypic characters can be used for taxonomic purposes to separate genera and for evolutionary analyses.

Up to now, karyotypes of 16 species (about 2/3 of total) of Megophryinae have been obtained (Table 1). They can be generally divided into two types on the basis of the numbers of large and small chromosomes: 6+7 and 5+8. *Brachytarsophrys feae*, *B.* sp. nov., *Megophrys shapingensis* (previously *Atympanophrys shapingensis*), *M. giganticus*, *M. nasuta*, *M. monticola* (synonym of *M. montana*), *M. daweimontis* sp. nov., and *M. palpebralespinosa* have the 6+7 pattern, whereas *M. parva*, *M. lateralis*, *M. minor*, *M. boettgeri*, *Ophryophryne microstoma* and *O. pachyprocta* have the 6+7 pattern. Although *M. kuatunensis* has 6 large and 6 small pairs it belongs to the 6+7 type. *M. omeimontis* has 5 large, 1 moderate and 7 small pairs. Thus it seems this pattern is intermediate between 5+8 and 6+7 types. In morphology *M. omeimontis* is similar to species which share the 5+8 pattern.

Morescalchi (1973, 1977) thought that the 5+8 pattern was derived from the 6+7 pattern through the lose of a fragment from one of its large chromosomes (usually No. 6).

The No. 6 chromosome of *M. omeimontis* is peculiar (see Fig. 3d). It seems that this chromosome is easily broken at the site of secondary constriction in the short arm and becomes 5+8 if the separated part is lost. This hypothesis supports the conclusion of Morescalchi with respect to the evolution of karyotypes in Pelobatidae. The karyotype of *M. omeimontis* should be considered derived from the 6+7 type. We suggest that one of the large chromosomes transferred a fragment to a small chromosome and the large one became small. The small one (T or ST chromosome) became the No. 6. The transferred fragment is the satellite on the short arm of No. 6 and it retains the former body type in morphology because the fragment is not lost. If the chromosomal fragment was lost, then the karyotype would have resembled the 5+8 pattern.

All the large sized species have 5+8 type karyotypes. While the species which are generally small sized have two karyotypic patterns. *Megophrys palpebralespinosa* and *M. daweimontis* sp. Nov. Have a 5+8 pattern, and the other *Megophrys* and *Ophryophryne* species have a 6+7 pattern.

It is interesting that *M. giganticus*, *Atympanophrys shapingensis*, *Brachytarsophrys* species, *M. nasuta* and *M. monticola* have not only the same type (5+8) of karyotype, but also similar morphological characters and habitats; i. e. very large bodies; very large, wide and flat heads; a hidden tympanum, round digital tips; and usually sit under stones at the edges of streams during the breeding season (Bourret, 1942; Inger, 1954; 1966; Liu and Hu, 1961; Tian et al., 1986; Yang et al., 1991).

The Classification of Megophryinae

The megophryinae is currently divided into 4 genera: *Atympanophrys*, *Brachytarsophrys*, *Megophrys*, *and Ophryophryne*. Some recent researchers think that the genus *Atympanophrys* is not valid because its main distinguishing character (no tympanum) is wrong and this genus should be a synonym of *Megophrys* (Fei et al, 1990). *A. shapingensis* has a unique karyotype as well as other morphological characters. This genus has a 5+8 karyotype, relatively large sized body, relatively flat and wide head, a hidden tympanum and round digital tips. It occurs always under stones at the sides of streams.

Brachytarsophrys includes three species (Rao and Yang, in press): *B. feae* (previously *M. feae*), *B. carinensis* and a new species from China. *B. feae* and the new species have 5+8 karyotypes. It is probable that *B. carinensis* also have this kind of karyotype. All the species in this genus have very large sized bodies, widened and flattened heads, a hidden tympanum, shortened legs and horny-bearing eyelids. They occur under stones at the sides of streams. In our opinion, this genus is valid.

Ophryophryne has three species: *O. microstoma*, *O. pachyprocta* and *O. poilani*. The former two have 6+7 karyotypes. All the species in this genus have small bodies, marrow mouths, small high heads, a dis-

tinct tympanum, and tubercled eyelids. They are usually found on grass and leaves beside very small streams (this is unique among the Megophryinae). This genus is valid, and it is related to the species of *Megophrys* which have 6+7 karyotypes.

Megophrys is a large genus, with about 22 species. Their body forms are varied. The karyotype patterns differ. *M. giganticus* is very large, it has 5+8 karyotype, an obviously wide and flat head, a hidden tympanum, and round digital tips. The habitats and habits for this species are similar to those of *Brachytarsophrys* and *A. shapingensis*. *M. montana* (including former *M. monticola*), and *M. nasuta* all have 5+8 karyotypes, broad heads, relatively short legs, an indistinct or hidden tympanum similar to *Brachytarsophrys*. However, they have elongate free dermal flaps on the snout and long soft horn-like appendages on the eyelids. Their habitats and habits are similar to *Brachytarsophrys*. The genus *Megophrys* was first described on the basis of the type, *M. montana*.

Megophrys palpebralespinosa and *M. daweimontis* sp. nov. have 5+8 karyotypes, a small sized body tubercled eyelids, reddish inner palm tubercles, and widened and sucker-like digital tips. They are usually found on grass at the sides of small streams. The rest of *Megophrys*, including *M. lateralis*, *M. minor*, *M. kuatunensis*, *M. parva* and *M. boettgeri* have 6+7 karyotypes, are generally moderate or small in body size, with regular body forms and head, a distinct tympanum, no tubercles on the eyelids, and widened and sucker-like digital tips. They are found on stones or roots at the sides of streams or rivers. Although *M. omeimontis* seems to be intermediate between the 5+8 and 6+7 karyotype, it is similar to *M. lateralis* *M. parva* in morphology, habitats and habits.

Thus, the species of *Megophrys* may be divided into four groups according to their karyotypes, morphology, habitats and habits: 1). *M. montana* and *M. nasuta*; 2). *M. giganticus*; 3. *M. palpebralespinosa* and *P. daweimontis* sp. nov.; 4). *M. lateralis*, *M. omeimontis*, *M. parva*, *M. minor*, *M. kuatunensis*, *M. boettgeri* and probably including *M. nankianensis*, *M. kempii*, *M. baluensis*, *M. aceras*, *M. brachykolos* and *M. robusta*.

Because of these differences in karyotype and morphology, we recognize a new genus for the *M. lateralis* and the *M. omeimontis* group. *Megophrys* should be restricted to the *M. montana* and *M. nasuta* group. The *M. lateralis* group is generally small in size, the heads are not wide, tympanum is present, legs long, digital tips flat and sucker-like, temporal fold thin and bent, and a 6+7 karyotype. Frogs of this group are usually found sitting on rocks next to rivers. The *M. montana* group is somewhat similar to *Brachytarsophrys* in cytology, morphology and habitats: 5+8 karyotype, very large body, wide and flat head, a hidden tympanum, straight and thick temporal fold, transverse shoulder groove, and short legs; but it is different in lacking tubercles on the upper eye-lid.

As for *M. giganticus*, it is narrowly distributed in Jingdong and Yondge, Yunnan. It is similar in morphology to *A. shapingensis* with digital tips round, hidden tympanum, wide and flat hear, straight and thick temporal folds, and 5+8 karyotype. *M. giganticus* is different than *Brachytarsophrys* and the *M. montana* group in having much longer legs, lacking any elongated tubercle on the upper eye-lid and no transverse groove on the shoulder. We suggest that *M. giganticus* should be placed in the genus *Atympanophrys* along with *A. shapingensis*.

Megophrys palpebralespinosa and *M. daweimontis* sp. nov. are more similar to the *M. lateralis* group even though they have 5+8 karyotypes. But they have tubercles on the upper eye-lid and reddish inner palm tubercles, which is different from the *M. lateralis* group. These species may be derived from an ancestor with 6+7 karyotype.

Based on the above information, Megophryinae is reviewed below:

1. The genus *Brachytarsophrys* is valid and it contains three species: *B. carinensis*, *B. feae* and *B.* sp. nov. (Rao and Yang, 1996). The type species if *B. feae*.

2. The genus *Ophryophryne* is valid and it contains three species: *O. microstoma*, *O. pachyprocta* and *O. poilani*. The type species is *O. microstoma*.

3. The genus *Megophrys* should be confined to the *M. montana* group. It includes two species: *M. montana* and *M. nasuta*. The type species if *M. montana*.

4. *Atympanophrys* should be re-named and its diagnosis should be revised. The genus should include *M. giganticus*.

5. A new genus *Panophrys* should be recognized for the *Megophrys lateralis* and *M. omeimontis* group. Its type species is *P. omeimontis*. This new genus can be distinguished on the basis of small size, tympanum present, head not very wide and flat, long legs, upper eye-lids tubercles absent (except for *P. palpebralespinosa* and *P. daweimontis*), temporal fold thin and bent, digital tips flat and sucker-like. They are usually found on rocks.

As for the relationships of this subfamily, two branches are recognized (Fig. 1): one for *Brachytarsophrys*, *Megophrys*, and *Atympanophrys* and another for *Panophrys* and *Ophryophryne*. The two

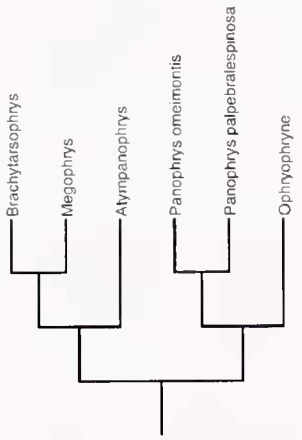

Figure 1. Evolutionary tree of Megophryinae.

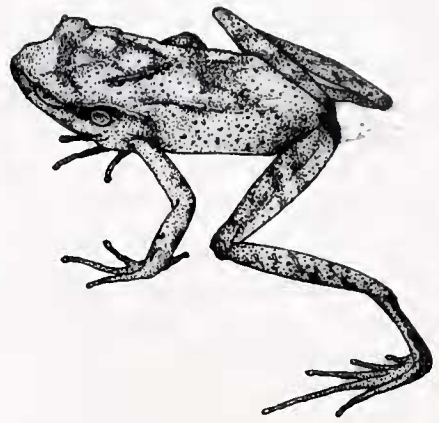

Figure 2. *Megophrys daweimontis* sp. nov. female X 1.

branches diverged early in megophryine evolutionary history. Both branches share almost the same distribution.

Description of a New Species

The specimens collected from Mt. Dawei, Pingbian County, southeast Yunnan are similar to specimens of *M. brachykolos, M. parva* and *M. longipes.*, but they have horn-like tubercles on the eyelids and the head is not flattened. In this character they can be distinguished from *M. parva.* The Mt. Dawei specimens have relatively long legs where the heals can overlap when the flexed legs are held at right angles to the body axis. The specimens have markings on the backs for heads and trunks, which are different from those of *M. brachykolos.* These markings are similar to those similar to those of *M. longipes.* According to Taylor (1962), *M. longipes* has rudimentary webs between the toes, no metatarsal tubercles, upper eyelids with small horn-like tubercles, and a body length of 47 mm in males and 65 mm in females; whereas the specimens from Mr., Dawei lack metatarsal tubercles, have smaller tubercles on the eyelids, lack webs between the toes, and are shorter (37 mm in males and 45 mm in females). Therefore we recognize the specimens from Mt. Dawei as a separate species.

Megophrys daweimontis sp. nov. (Fig. 2)

Holotype: KIZ 93088, adult male, collected from Mt. Dawei, Pingbian County, southeast Yunnan, China., altitude 1900 m., July, 1993.

Paratypes: 17 adult males (KIZ 93069-KIZ 93085) and 3 adult females (KIZ 93086, KIZ 93087, KIZ 93089) collected at the same place and time as the holotype.

Diagnosis: A small tubercle on outer edge of upper eyelid; vomerine teeth present; snout projecting beyond jaw; tibiotarsal articulation reaching tip of snout; vocal sac in male; toes without rudiment of web; an inner palm tubercle and very small outer palm tubercle, a reddish inner metatarsal tubercle; body length from shout to vent 34-37 mm in males and 40-46 mm in females.

Description: Tongue pyriform; vomerine teeth present; head slightly wider than long, depressed; snout very short; interorbital space concave; tympanum distinct, round. Arms long and slender, the first finger extending beyond second; palm tubercles reddish, the inner tubercle is very large and the outer very small. Legs long, the distal end of femur reaching shoulder; tibiotarsal articulation reaching tip of snout; toes slender, swollen at tips, completely without webbing. Superarticular tubercles lacking; a reddish outer metatarsal tubercle.

Skin smooth above, with small warts on flanks and sides of body; two pairs of delicate oblique folds on the scapular region extending posteriorly to waist, a pair of folds on the back-side; fold extending from eye above tympanum to shoulder: upper eyelid with very small tubercle on outer edge; lower surfaces smooth.

Olive-brown above, a triangular marking between eyes, followed with V-shaped marking above shoulder, or X-shaped marking on the back of trunk; black marking beneath the vent and behind heel; transverse lines above thigh; inner lower surface of legs reddish.

Measurements of holotype are: 33.5 mm snout-vent; 12.5 mm head length; 12.5 mm head width; 52.5 mm hind leg; 18 mm tibia.

Distribution: Known only from type locality.

Variation: Body length 33-37 mm in males and 40-46 mm in females.

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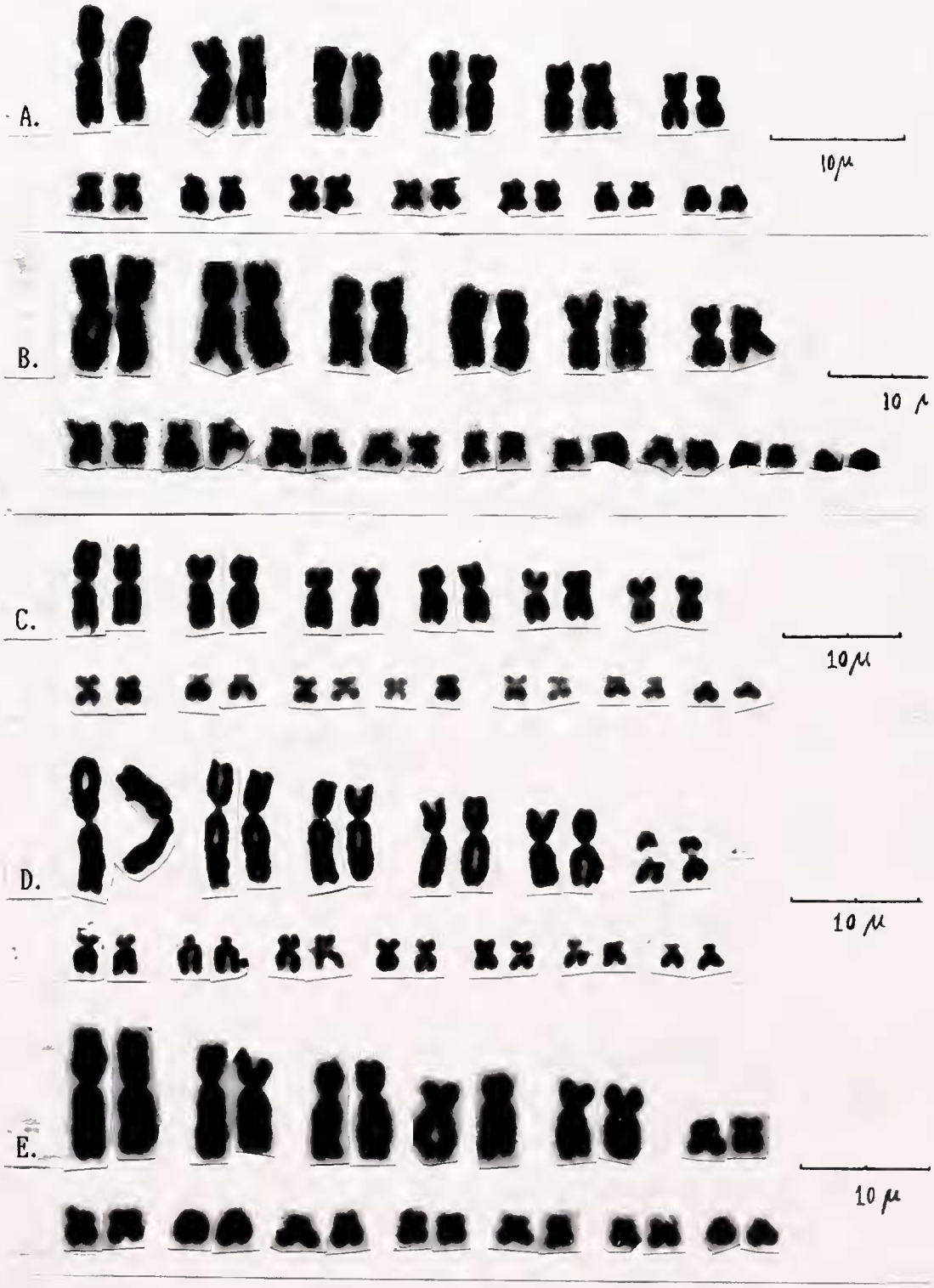
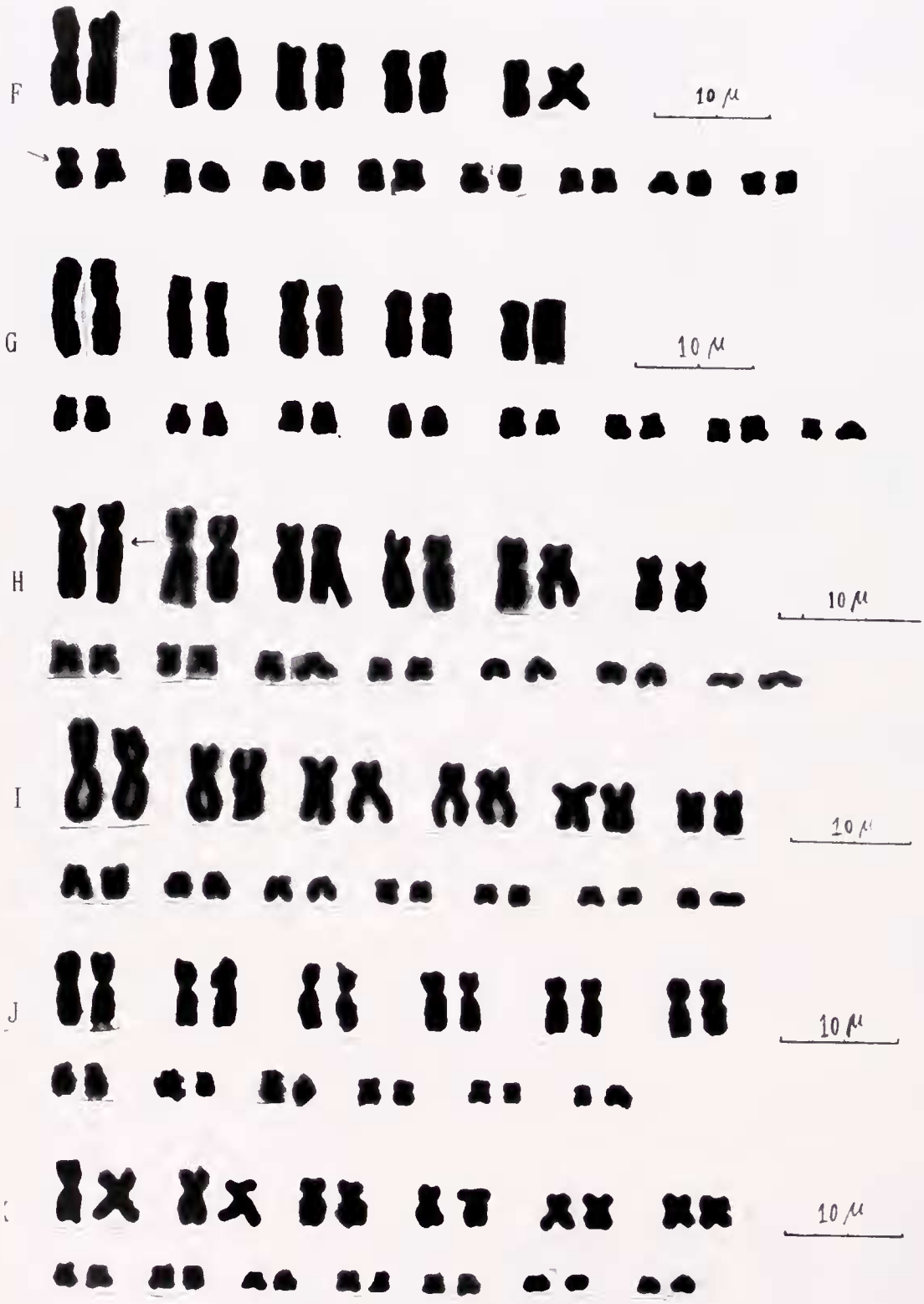
Appendix I

Figure 3. The karyotypes of Megophryinae species: A-B. *Megophrys minor;* C. *M. lateralis* (Tengchong); D. *M. omeimontis;* E. *M. giganticus;* F. *M. daweimontis* sp. nov.; G. *M. palpebralespinosa;* H. *M. parva;* I. *M. lateralis* (Hekou); J. *M. kuatunensis;* K. *Ophryophryne microstoma.*

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